REMARKS

This is in response to the Office Action dated July 2, 2007. Claims 17-37 were acted upon by the Examiner. Claim 17 has been amended. No claims have been canceled or added. Accordingly, claims 17-37 are presented for examination.

Amendments to the Specification

Two new paragraphs have been added to the end of the Background section to correlate with the presently pending claims. The new paragraphs are supported by claims 1, 4, 7, and 8 as originally filed. The more commonly used term "differentiable" has been substituted for the term "deconvariable" as used in originally filed claim 4. No issue of new matter is seen by this amendment.

Amendments to the Claims

Claim 17 is amended to specify that the signaling hairpins do not include the capture probe. Literal support for this limitation is found, for example, at page 5, lines 1-2 where it is specifically states that "It [a signaling hairpin] need not, and in preferred embodiments does not, function as a probe." Additionally, support is found in original claim 4, now included (with original claims 1, 7 and 8) in the specification, as permitted. Claim 17 is also amended to specify that the physical or chemical change in environment that disrupts signaling hairpins is "controllable". Literal support for this limitation is found, for example, at page 4, lines 30-31 where it states "a flanking affinity pair whose interaction can be disrupted by a controllable physical or chemical change in its environment." The phrase "for said at least one sequence" was added after the recitation of "a capture probe" to make clear that it is the capture probe that hybridizes to the "at least one sequence." No issues of new matter are seen by these amendments.

Summary of the Presently Claimed Invention

The presently claimed invention is directed to a hybridization assay for at least one of a multiplicity of nucleic acid sequences in an analyte that uses a unique and nonobvious coding

scheme. As amended, claim 17 (the only independent claim pending) comprises the steps of (a) contacting the analyte with a mixture of encoded microcarriers, (b) forming a distributed array of said microcarriers; (c) determining which microcarriers have capture probes hybridized to said at least one nucleic acid sequence of said analyte; and (d) optically decoding the microcarriers having hybridized capture probes to identify said at least one nucleic acid sequence. The hybridization assay of the presently claimed invention uses unique and non-obvious microcarriers that have immobilized on their surface (i) a capture probe for the at least one sequence, and (ii) a coding scheme comprising a plurality of signaling hairpins ***that are not capture probes***. The signaling hairpins include affinity pairs that are disruptable by a controllable physical or chemical change in a condition of its environment. In the presently claimed invention, the disruptions result in fluorescent signals which are used to differentiate the microcarriers.

Discussion of Examiner's Claim Interpretation

The Examiner has interpreted "the capture probes" to be signaling hairpins. The Examiner has also pointed out "that there is no requirement in claim 17 that capture probe be different or separate from the signaling hairpin." As discussed above, claim 17 has been amended to specifically recite that the signaling hairpins are not capture probes.

The Examiner has interpreted "distributed array," which appears in element (b) of claim 17, as an array distributed on a planar surface, including a linear array. Indeed, the specification teaches very broadly that a distributed array is any array in which the microcarriers can be individually addressed. See, for example, page 7, lines 4-5, wherein utilization of known methods and systems is specified, a number of which are described on page 2, including planar arrays (lines 2-3), immobilizing on the ends of strands in a bundle (lines 10-13), and a fluorescence-activated cell sorter (line 16). See also page 14, which specifies using any assay method utilizing a distributed array (lines 26-27), a number of which are described on that page, including settling onto a planar surface (lines 9-10), fixing between two planar surfaces in a flow cell (lines 12-13, and see additionally page 8, lines 10-11), capturing onto the ends of fibers of a fiber-optic bundle

(lines 17-18), passing beads through a flow cytometry system (lines 22-23), and distributing into a linear array in a capillary tube (lines 24-24). Accordingly, it is understood that the Examiner intends that "distributed array" have its broadest meaning, as is intended.

Discussion of Section 103 Rejections

Claims 17-22, 24-31, 33-36 are rejected under 35 U.S.C. §103(a) as being unpatentable over Bruchez, Jr. et al. (US Patent No. 6,500,622) in view of Chee et al. (WO 01/46675). Claims 23 and 32 are rejected under 35 U.S.C. §103(a) as being unpatentable over Bruchez, Jr. et al. in view of Chee et al. as applied to claims 17-18 and in further view of Bonnet et al. (Proc Natl. Acad. Sci. 96:6171-76 (1999)). Accordingly, each of these rejections depends upon the combination of Burchez et al. and Chee et al.

"To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." MPEP § 2143. Here, the combination of Burchez et al. and Chee et al. does not teach or suggest all the claim limitations of claim 17 (and thus the limitations of claims 18-32 which depend from claim 17).

The combination of Bruchez et al. and Chee et al.
<u>does not teach or suggest the claimed coding scheme</u>

Regarding claim 17, the Examiner concludes that Bruchez et al. teaches all the elements of claim 17 except for "a mixture of encoded microcarriers" recited in step (a) of the claim. The Examiner turns to Chee et al. for that limitation.

The asserted combination does not teach or suggest the scheme for coding and decoding microcarriers of claim 17. In multiplex assays utilizing microcarriers with immobilized capture probes, there are two tasks that must be accomplished. First, one must be able to determine whether or not the microcarriers have captured their targets. This may be done in a number of ways, one of which is to use a molecular beacon probe to signal capture. This and other ways to determine that capture has occurred are disclosed by Bruchez et al. at, for example, column 2, lines 47-49. Second, you must be able to distinguish among the microcarriers with different capture probes. Because there are only a limited number of fluorophores that can be used with molecular beacons, the beacons alone can only be used to distinguish among seven or eight targets. See, for example, this application at page 2, lines 18-20. That is the problem sought to be solved by Bruchez et al., Chee et al., and the instant application. The solutions of all are to encode the microcarriers so that each has a code that identifies which capture probe it carries. Each, however, uses a different coding scheme to distinguish among the captured microcarriers.

As indicated by the citations below, Bruchez et al. relies on semiconductor nanocrystals to differentiate the beads carrying different probes.

- "The large number of distinguishable semiconductor nanocrystal labels allows for the simultaneous analysis of multiple labeled target polynucleotides, along with multiple different encoded bead conjugates." (column 3, lines 3-5).
- "'Multiplexing' herein refers to an assay or other analytical method in which multiple probe polynucleotides can be assayed simultaneously by using more than one SCNC [semiconductor nanocrystal], each of which has at least one different fluorescence characteristic ...." (column 13, lines 49-52).
- "Microspheres can be spectrally encoded through incorporation of SCNC's. The desired fluorescence characteristics may be obtained by mixing SCNC's ...." (column 16, lines 10-12).

One particular problem with the Bruchez approach is that it involves complicated manufacturing.

The solution proposed by Chee et al. is unlabeled oligonucleotides as labels for different beads carrying different probes. For example, one may use combinations of five different oligonucleotides as the code. Following a determination that a bead has captured some target, it is decoded by subjecting it to five serial hybridizations reactions with labeled complementary oligonucleotides (they can all be the same color) to decode the bead and thereby identify which capture probe garnered a product. This approach, similar to the method discussed in the background section of the instant application at page 3, lines 4-16, is methodically complicated, cumbersome and expensive.

While the claimed method is similar to the methods of Bruchez et al. and Chee et al. regarding use of microcarriers, capture probes and determining whether a bead has made a capture, it is uses a fundamentally different and non-obvious scheme for coding of the microcarriers to identify microcarriers that have captured a target and thereby identify which capture probe has been successful. Unlike Bruchez et al. and Chee et al., claim 17 does not recite a coding scheme that uses nanocrystals or unlabeled oligonucleotides that are subjected to serial hybridizations. Rather, claim 17 recites a coding scheme that uses signaling fluorescent hairpins that open and signal at controllable physical or chemical changes in the environment. For example, one hairpin that has a red fluorophore may signal at 50 °C, another at 60 °C, and yet another at 70 °C. A combination of a few spectrally deconvolvable fluorophores and a few differentiable conditions is the code for each microcarrier.

The references do not teach or suggest the claimed method. Bruchez et al. recognizes that molecular beacon (labeled hairpin) probes can be used as the capture probes, but nonetheless teaches a coding scheme that does not use labeled hairpins. Chee et al. recognizes that a stable of oligonucleotides of different sequences can be used as a coding scheme, but only serial hybridizations are used for detection of the code. Thus, neither reference, nor the references in combination, suggests the claimed coding scheme, which involves neither complicated manufacturing nor complicated processing.

As explained above, the proposed combination of references fails to teach all the limitations of claim 17 (and the limitations of claims 18-37 which depend from claim 17), and thus does not meet the requirements necessary to establish a *prima facie* case of obviousness.

The Examiner's citations to Bruchez et al. do not
teach or suggest the presently claimed coding scheme

Applicants agree with the Examiner that Bruchez et al. teaches multiplex assays for at least one of a multiplicity of target nucleic acids using encoded beads having conjugated thereto different capture probes and contacting an analyte with a mixture of these encoded beads. However, because Bruchez et al. is directed to a fundamentally different coding scheme for determining which microcarriers captured targets, as discussed above, Applicants respectfully submit that many of the Examiner's assertions that Bruchez et al. discloses certain recitations of claim 17 are inaccurate. In order to highlight the differences between Bruchez et al. and the presently claimed invention, some of these inaccuracies are discussed below.

Contrary to the Examiner's assertion on page 5 of the Action, Bruchez et al. does not teach a plurality of *signaling* hairpins. The passages relied upon by the Examiner state that one can have on the same substrate two different capture probes, which can be differentiated from one another by location, as was known for fixed arrays, or, if the substrate is a bead, by labeling the amplified targets with differently colored fluorophores. So, for 50 targets, one could use 25 beads, each with two unlabeled capture probes. Because one can not differentiate 50 targets with fluorophores, the coding scheme for 25 beads would still be needed, and for Bruchez et al. that is the use of embedded semiconductor nanocrystals to identify which bead recovered target. The fluorophore on the target would only identify which of two possible targets hybridized to the bead. In contrast, claim 17, as amended, recites that the probes and signaling hairpins are different.

On page 5 of the Action, the Examiner asserts that Bruchez et al. teaches the use of four molecular beacon probes that meets the coding scheme recited in claim 17 (a)(ii). Applicants respectfully submit that this is incorrect. At the beginning of Example 2 (from which the Examiner cites) at column 38, lines 59-62, Bruchez et al. teaches that each molecular beacon capture probe was labeled with fluorescein and the quencher DABCYL. Each of the four capture probes was then conjugated to one of the semiconductor nanocrystal-encoded microcarriers. The molecular beacons only signaled hybridization, not which hybridization. The embedded nanocrystals signaled the identity of the bead that gave the fluorescein emission, thereby identifying which of the four identically labeled molecular beacon capture probes had been successful. Figure 2 of Bruchez et al. is to the same effect. It shows a molecular beacon capture probe attached to an "Encoded Microsphere." "The invention described here is attachment of MBs [molecular beacon capture probes] onto encoded microspheres dyed with one or more different kind[s] of SCNC's." (column 30, lines 62-64). Again, claim 17 recites a plurality of signaling hairpins that are not captured probes.

Moreover, the Examiner's assertion on page 6 of the Action is incorrect that Figure 2 of Bruchez et al. shows a signaling hairpin opening in response to a chemical change in a condition of its environment. Figure 2 shows a capture probe opening due to hybridization with its target. This is not an environmental change in the context of the instant application. Claim 17 is amended to make that clearer by specifying that the chemical change of the claim is a "controllable" change, which is not the case for possible target. Accordingly, the molecular beacon capture probes of Bruchez et al are fundamentally different from the signaling hairpins as used in claim 17.

As the Examiner notes on page 6 of the Action, Bruchez et al., at column 35, lines 9-30, teaches the use of two molecular beacon capture probes for two SNP alleles. The Examiner, however, is incorrect in concluding that the next paragraph teaches discriminatory disruption by a change in salt and temperature. That paragraph, column 35, lines 31-54, states that each

molecular beacon capture probe is immobilized on one of the spectrally encoded (that is, SCNC-encoded) beads; then target-containing solution is combined with a solution containing both beads, and, to the extent each SNP is present, its complementary capture probe binds and opens. There is no teaching of disruption of one signaling hairpin or even one molecular beacon capture probe at a first controllable environmental condition and disruption of a second signaling hairpin or even a second molecular beacon probe at a second controllable environmental condition.

Furthermore, the Examiner's citation of Bruchez et al. column 24, lines 48-59 against recitation (a)(ii) on page 7 of the Action is incorrect. Line 25 of that column begins a section titled "Labels" and begins, "Labels useful in the inventions described herein include any substance which can be detected in association with the substrate when the molecule to which the label is attached, directly or indirectly, is ***hybridized to a polynucleotide*** which is attached to the substrate." (column 24, lines 26-30) (emphasis added). Again, this passage does not disclose the encoded-hairpins coding scheme of limitation (a)(ii).

Finally, the Examiner's citation on page 7 of the Action to column 31, lines 30-51 of Bruchez et al. against the coding scheme of claim 17, step (a) is incorrect. Line 30 begins, "Different MBs [molecular beacon capture probes] can be conjugated onto SCNC-encoded microspheres (any size) of different colors or color combinations for multiplexing." In Bruchez et al., the coding scheme for the microparticles is SCNC, not the fluorophores, which are to signal hybridization. In contrast, the coding scheme of claim 17 uses separate signaling hairpins *that are not used for capturing* to signal which microcarriers have captured the target.

Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Conclusion

In view of Applicant's claim amendments and the arguments presented above, the present application is believed to be in condition for allowance and an early notice thereof is earnestly

solicited. If any issues remain, Applicants request that the Examiner contact the undersigned before issuing another action.

Respectfully submitted,

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